

Pre-Treatment Procedures

- Animal health procedure: all animals received a clinical examination for ill-health on arrival and a veterinary clinical examination during the acclimatization period.
- 5 ▪ Acclimatization period: at least 3 weeks between animal arrival and start of treatment.

Experimental Design

- Allocation to treatment groups was performed during the acclimatization period using a random allocation procedure based on body weight classes.
- 10 ▪ Animals were assigned to the treatment groups shown in Table 1. The dose levels administered were shown in Table 2.

Administration of the Test/Control Articles

Group 1 and 2 Animals

- Method of administration: injection in the left inguinal lymph node.
- 15 Animals were lightly anaesthetized before each administration by an intramuscular injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France). The same lymph node was injected on each occasion (left side). Each injection was followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France).

Group 3

- Route: subcutaneous.
- Method of administration: bolus injection using a sterile syringe and needle introduced subcutaneously. Four injection sites were used followed by a local disinfection with iodine (Vétédine® - Vétoquinol, Lure, France).
- 25 Animals were also lightly anaesthetized before each administration by an intramuscular injection of ketamine hydrochloride (Imalgene® 500 - Merial, Lyon, France) in order to be under the same conditions as groups 1 and 2 animals.

Four injection sites in the dorsal cervical/interscapular regions were used as shown in Table 3.

▪ **ELISPOT Analysis**

An ELISPOT assay was used in order to assess the cell mediated immune response generated in the monkeys in the various treatment groups. In particular, an ELISPOT IFN γ assay was used in order to measure IFN γ production from T lymphocytes obtained from the monkeys in response to gp100 antigens.

10 Materials and Methods

Plates: MILLIPORE Multiscreen HA plate / MAHA S45.10 (96 wells).

Capture antibodies: MABTECH monoclonal anti-IFN γ antibodies/G-Z4 1 mg/mL.

Detection antibodies: MABTECH monoclonal anti-IFN γ antibodies/7-B6-1 15 biotin 1 mg/mL.

Enzyme: SIGMA, Extravidin-PA conjugate/E2636

Substrate: BIORAD, NBT/BCIP - Alkaline phosphatase conjugate substrate kit/ref: 170-64 32.

Coating

20 Place 100 μ L per well of capture antibodies at 1 μ g/mL diluted at 1/1000 in carbonate bicarbonate buffer 0.1M pH 9.6 into the multiwell plate. Incubate overnight at 4°C. Wash 4 times in 1X PBS.

Saturation

Place 200 μ L per well of RPMI supplemented with 10% FCS, non essential 25 amino acids, pyruvate, Hepes buffer and Peni-Strepto. Incubate 2 hours at 37°C.

Test

Cells from the immunized animals are tested against (a) medium alone; (b) pooled peptides at a concentration of 1 mg/mL; and (c) a non specific

stimulus (PMA-Iono). The pooled peptides used in this Example to stimulate IFN- γ production were derived from gp100 and are illustrated in Tables 4 to 7. The final volume of each sample is 200 μ L. Incubate 20 hours at 37°C.

5 Wash 4 times in 1X PBS and 0.05% Tween 20.

Detection

Place 100 μ L per well of detection antibodies at 1 μ g/mL diluted in 1/1000 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 2 hours at room temperature. Wash 4 times in 1X PBS and 0.05% Tween 20.

10 **Reaction**

Place 100 μ L per well of Extravidin-PA conjugate diluted 1/6000 in 1X PBS, 1% BSA and 0.05% Tween 20. Incubate 45 minutes at room temperature.

Wash 4 times in 1X PBS and 0.05% Tween 20.

Substrate Addition

15 Place 100 μ L per well of substrate previously prepared. For example, for 1 plate, prepare: 9.6 mL of distilled water, 0.4 mL of 25X buffer, 0.1 mL of solution A (NBT) and 0.1 mL of solution B (BCIP). Incubate 30-45 minutes at room temperature. Wash in distilled water. Dry and transfer to a plastic film. The number of spots are counted using a Zeiss image analyzer. Each
20 spot corresponds to an individual IFN- γ secreting T cell.

Results

The animals that tested positive on the ELISPOT analysis are shown in Figures 1-4. Overall, the results demonstrate that of the animals tested, 2
25 out of 2 (i.e. 100%) of the animals that received the intranodal administration of the gp100 antigen, and 2 out of 4 (i.e. 50%) of the animals that received the subcutaneous administration of the gp100 antigen had a positive cell mediated immune response.

ELISA Analysis

The ELISA was performed utilizing standard methodology known in the art. Briefly, the human gp100 ("hgp100"; produced in Baculovirus) was diluted in 5 coating buffer (carbonate-bicarbonate, pH9.6) and added to 96 wells at 0.5ug/well. Plates were placed at 4°C overnight. Plates were then washed and blocking buffer (phosphate buffered saline/0.5% Tween 20/1.0% BSA, pH7.2) was added for 2 hours at 37°C. The plates were then washed and the sera was diluted in dilution buffer (phosphate buffered saline/0.5 % 10 Tween 20/ 0.1 BSA, pH7.2). For this study, monkey sera was diluted to 1:800 and "7" serial 3 fold dilutions were done for each sample tested. The human sera controls were diluted to 1:50 in dilution buffer and "7" serial 2 fold dilutions were performed. Each dilution was done in duplicate. The plates were incubated a further 2 hours at 37°C. The plates were washed 15 and the horse radish peroxidase (HRP)-conjugated anti-human secondary antibody (anti-human Ig whole antibody from sheep (Amersham Life Science, NA933)) diluted 1:100 in dilution buffer was added to the wells and incubated for 1 hour at 37°C. The plates were washed and OPD (o-phenylenediamine dihydrochloride) substrate with H₂O₂ in substrate buffer 20 (50mM phosphate/25mM citrate, pH 7.2) was added to the wells. For a kinetics ELISA, the plate was read repeatedly (2 minute intervals for 15 minutes) unstopped (without "stop" buffer). Plates were read at 450nm.

Results

25 The results of the above experiment are presented in Table 8 and in Figure 5. The animals of group 2 received intranodal injections of ALVAC(2)-gp100(mod) followed by boosts with the modified gp100 peptides 209(2M) and 290(9V); the animals in group 3 received a subcutaneous

injection of the ALVAC(2) construct followed by peptide boosts; the animals in group 1 received intranodal injections of saline as a control.

As can be seen from Figure 5, intranodal injection of the antigens induced a humoral response that was much greater than when the antigen 5 was injected subcutaneously.

In summary, the results of this Example demonstrate that intranodal injection of a tumor antigen induces both a humoral and cell mediated response that is much greater than when the tumor antigen is injected by the conventional subcutaneous route of administration.

10 While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the 15 appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

TABLE 1

Group Number	Route of administration	Treatment days and compound administered	Number of Animals
1	Intranodal	Saline (NaCl 0.9%): days 28, 42, 56 Then 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
2	Intranodal	<u>ALVAC(2) - gp100 mod. days 28, 42, 56</u> *mgp100 peptides: days 70, 71, 72, 73, 74 Then 84, 85, 86, 87 and 88	4
3	Subcutaneous	Saline (NaCl 0.9%): day 1 ALVAC(2) - gp100 mod. days 28, 42, 56 *mgp100 peptides: days 70 and 84	4

*209(2M)-IMDQVPFSY: 290(9V) YLEPGPVTV

5 ▪ Group 1 animals (control) received the control article (saline for injection (NaCl 0.9%)).
 ▪ Group 3 animals received the control article (saline for injection (NaCl 0.9%)) on day 1 only.

36
TABLE 2

Group Number	Dose level	Dose volume (ml/administration)
1	Saline (NaCl 0.9%): 0	0.250
2	Dose: 0.25×10^{14} CCID 50 ALVAC (2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID50 Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100µg each)	0.250 0.2
3	Saline (NaCl 0.9%) ALVAC(2) - gp100 mod: $0.25 \times 10^{7.4}$ CCID 50 Dose: 200 µg (Total) of peptides IMDQVPFSY (209(2M)), and YLEPGPVTV (290(9V)) (100µg each)	0.250 0.250 0.2

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TABLE 3

Days	Sites used
1 and 28	lower left
42	upper left
56	upper right
70	lower left
84	lower right

TABLE 4

Peptide Pool #1

Peptide	Sequence	SEQ.ID.NO.
1329	HLAVIGALLAVGATK	SEQ.ID.NO.3
1330	GALLAVGATKVPRNQ	SEQ.ID.NO.4
1331	VGATKVPRNODWLGV	SEQ.ID.NO.5
1332	VPRNQDWLGVSRLQR	SEQ.ID.NO.6
1333	DWLGVSRLTKAWN	SEQ.ID.NO.7
1334	SRQLRTKAWNRLQYP	SEQ.ID.NO.8
1335	TKAWNRLQYPFWTEA	SEQ.ID.NO.9
1336	RQLYPEWTEAQRLRDC	SEQ.ID.NO.10
1337	EWTEAQRLDCWRGGQ	SEQ.ID.NO.11
1338	QRLDCWRGGQVSLKV	SEQ.ID.NO.12
1339	WRGGQVSLKVSNNDGP	SEQ.ID.NO.13
1340	VSLKVSNNDGPTLIGA	SEQ.ID.NO.14
1344	IALNFPGSQKVLPDG	SEQ.ID.NO.15
1345	PGSQKVLFDGQVIVW	SEQ.ID.NO.16
1346	VLPDGQVIVWNNTII	SEQ.ID.NO.17
1347	QVIWVNNTIINGSQV	SEQ.ID.NO.18
1348	NNNTIINGSQVWGGOP	SEQ.ID.NO.19
1349	NGSQVWGGQPVYPQE	SEQ.ID.NO.20
1350	WGQOPVYPQETDAD	SEQ.ID.NO.21
1351	VYPQETDADACIFPDG	SEQ.ID.NO.22
1352	TDDACIFPDGGPCPS	SEQ.ID.NO.23
1353	IFPDGGPCPSGSWSQ	SEQ.ID.NO.24
1355	GSWSQKRSEVYWWT	SEQ.ID.NO.25
1356	KRSFVYVWKTWQYW	SEQ.ID.NO.26
1357	YVWKTWQYVWQVLGG	SEQ.ID.NO.27
1358	WGOYWQVLGGPVSGL	SEQ.ID.NO.28
1359	QVLGGPVSGLSIGTG	SEQ.ID.NO.29

39
TABLE 5

Peptide Pool #2

Peptide	Sequence	SEQ.ID.NO.
1360	PVSGLSIGTGRAMLG	SEQ.ID.NO.30
1361	SIGTGRAMLGTHME	SEQ.ID.NO.31
1362	RAMLGOTHIMETVYH	SEQ.ID.NO.32
1363	THTMETVYHRRGSK	SEQ.ID.NO.33
1364	VTVYHRRGSRSYVPL	SEQ.ID.NO.34
1365	RRGSRSYVPLAHSS	SEQ.ID.NO.35
1366	SYVPLAHSSAFTIT	SEQ.ID.NO.36
1368	AFTITDQVPPFSVSVS	SEQ.ID.NO.37
1369	DQVPPFSVSVQLRAL	SEQ.ID.NO.38
1370	SVSVSQLRALDQGNK	SEQ.ID.NO.39
1372	DGGGNKHFLRNQPLTF	SEQ.ID.NO.40
1373	HFLRNQPLTFALQLH	SEQ.ID.NO.41
1374	QPLTFALQLHDPPSGY	SEQ.ID.NO.42
1375	AQLLHDPSGTYLAED	SEQ.ID.NO.43
1379	DFGDSSGTLISRALV	SEQ.ID.NO.44
1380	STGLISRALVVTHTY	SEQ.ID.NO.45
1381	SRALVVVTHTYLEPGP	SEQ.ID.NO.46
1382	VVTHTYLEPGPVTAQV	SEQ.ID.NO.47
1383	LEPGPVTAQVVLQAA	SEQ.ID.NO.48
1384	VTAQVVLQAAIPLTS	SEQ.ID.NO.49
1385	VLOQAAIPLTSCGSSP	SEQ.ID.NO.50
1386	IPLTSCGSSPVPGTT	SEQ.ID.NO.51
1388	VPGTTDGHHRPTAEAP	SEQ.ID.NO.52
1389	DGHHRPTAEAPNTAG	SEQ.ID.NO.53
1390	TAEAPNTTAGQVPTT	SEQ.ID.NO.54
1392	QVPTTIEVVGQITPGQA	SEQ.ID.NO.55
1393	EVVGTTPGQAPTAEP	SEQ.ID.NO.56

40
TABLE 6

Peptide Pool #3

Peptide	Sequence	SEQ.ID.NO.
1394	TPGQAPTAEPSGTTS	SEQ.ID.NO.57
1395	PTAEPSTGTTSVQVPT	SEQ.ID.NO.58
1396	SGTTSVQVPTTEVIS	SEQ.ID.NO.59
1397	VQVPTTEVISTAPVQ	SEQ.ID.NO.60
1398	TEVISTAFQVQMP TAE	SEQ.ID.NO.61
1399	TAPVQMP TAE STGMT	SEQ.ID.NO.62
1400	MPTAESTGTM PKEKVP	SEQ.ID.NO.63
1401	STGMTFEKVPVSEVM	SEQ.ID.NO.64
1402	PEKVPVSEVMGTTLA	SEQ.ID.NO.65
1403	VSEVMGTTLAEMSTP	SEQ.ID.NO.66
1404	GTTLAEMSTPEATGM	SEQ.ID.NO.67
1405	EMSTPEATGM PTAEV	SEQ.ID.NO.68
1408	SIVVLSGTTAAQVTT	SEQ.ID.NO.69
1409	SGTTAAQVTTTEWVE	SEQ.ID.NO.70
1410	AQVTTTEWVETTARE	SEQ.ID.NO.71
1411	TEWVETTARELPIPE	SEQ.ID.NO.72
1412	TTARELPIPEPFGPD	SEQ.ID.NO.73
1413	LPIPEPEGPDASSIM	SEQ.ID.NO.74
1414	PEGPDASSIMSTESI	SEQ.ID.NO.75
1415	ASSIMSTESITGSLG	SEQ.ID.NO.76
1416	STESITGSLGPILLDG	SEQ.ID.NO.77
1417	TGSLGPILLDG TATLR	SEQ.ID.NO.78
1418	PILLDG TATLR LVKVRQ	SEQ.ID.NO.79
1419	TATLRLVVKRQVPLDC	SEQ.ID.NO.80
1420	LVKVRQVPLDCVLYRY	SEQ.ID.NO.81
1421	VPLDCVLYRYGSFSV	SEQ.ID.NO.82
1422	VLYRYGSFSVTL DIV	SEQ.ID.NO.83

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Table 7

Peptide Pool #4

Peptide	Sequence	SEQ.ID.NO
1424	TLDIVQGIESAEILQ	SEQ.ID.NO.84
1425	QGIESAEILQAVPSG	SEQ.ID.NO.85
1426	AEILQAVPSGEQDAF	SEQ.ID.NO.86
1427	AVPSGEQDAFELTVS	SEQ.ID.NO.87
1428	EGDAFELTVSCQGGL	SEQ.ID.NO.88
1429	ELTVSCQGGLPKEAC	SEQ.ID.NO.89
1430	CQGGLPKEACMEISS	SEQ.ID.NO.90
1431	PKEACMEISSPGCQP	SEQ.ID.NO.91
1432	MEISSPGCQPPAQRL	SEQ.ID.NO.92
1434	PAQRLLCOPVLPSPAC	SEQ.ID.NO.93
1435	CQPVLPLSPACQLVLH	SEQ.ID.NO.94
1436	PSPACQLVLHQILKG	SEQ.ID.NO.95
1437	QLVLHQILKGGSSTY	SEQ.ID.NO.96
1441	LADTNSLAVVSTQLI	SEQ.ID.NO.97
1442	SLAVVSTQLIMPQGE	SEQ.ID.NO.98
1443	STOLIMPQGEAGLGQ	SEQ.ID.NO.99
1444	MPGQEAGLGQVPLIV	SEQ.ID.NO.100
1445	AGLGQVPLIVGILLV	SEQ.ID.NO.101
1448	LMAVVVLASLIIYRRRL	SEQ.ID.NO.102
1450	YRRRLMKQDFSVPQL	SEQ.ID.NO.103
1451	MKQDFSVPQLPHSSS	SEQ.ID.NO.104
1452	SVPQLPHSSSHWLRL	SEQ.ID.NO.105
1453	PHSSSHWLRLPRIFC	SEQ.ID.NO.106
1454	HWLRLPRIFCSCPIG	SEQ.ID.NO.107
1455	PRIFCSCPIGENSPL	SEQ.ID.NO.108

TABLE 8

Monkey #	DAY (mOD/min)		
	0	57	68
1	3	5	2
2	4	6	12
3	7	6	10
4	7	6	8
5	5	9	20
6	11	8	10
7	11	23	51
8	7	30	70
9	1	7	5
10	2	6	6
11	3	7	14
12	6	9	15

We claim:

1. A method for inducing an immune response in an animal to a tumor antigen comprising administering an effective amount of a tumor antigen or a nucleic acid sequence encoding a tumor antigen to a lymphatic site in the animal.
2. A method according to claim 1 wherein the tumor antigen is selected from the group consisting of CEA, gp100, the MAGE family of proteins, DAGE, GAGE, RAGE, NY-ESO 1, Melan-A/MART 1, TRP-1, TRP-2, tyrosinase, HER-2/neu, MUC-1, p53, KSA, PSA, PSMA, and fragments and modified versions thereof.
- 15 3. A method according to claim 1 or 2 wherein the lymphatic site is a lymph node.
4. A method according to any one of claims 1 to 3 wherein the nucleic acid is selected from the group consisting of viral nucleic acid, bacterial DNA, plasmid DNA, naked/free DNA, and RNA.
- 20 5. A method according to claim 4 wherein the viral nucleic acid is selected from the group consisting of adenoviral, alphaviral and poxviral nucleic acid.
- 25 6. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of avipox, orthopox and suipox nucleic acid.
- 30 7. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of vaccinia, fowl pox, canarypox and swinepox nucleic acid.

8. A method according to claim 5 wherein the poxviral nucleic acid is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC nucleic acid.
5
9. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a vector.
10
10. A method according to claim 9 wherein the vector is a recombinant virus or bacteria.
11
11. A method according to claim 10 wherein the recombinant virus is selected from the group consisting of adenovirus, alphavirus and poxvirus.
15
12. A method according to claim 11 wherein the poxvirus is selected from the group consisting of avipox, orthopox and suipox.
20
13. A method according to claim 11 wherein the poxvirus is selected from the group consisting of vaccinia, fowlpox, canarypox and swinepox.
25
14. A method according to claim 11 wherein the poxvirus is selected from the group consisting of MVA, NYVAC, TROVAC, and ALVAC.
15. A method according to any one of claims 1 to 8 wherein the nucleic acid is contained in a cell.
26
16. A method according to any one of claims 1 to 14 wherein the tumor antigen or nucleic acid coding therefor is contained in a vaccine.
30

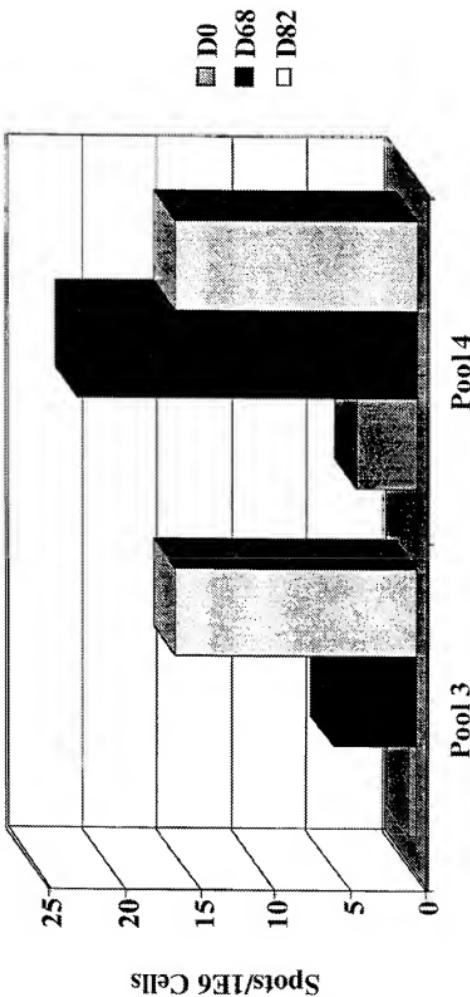
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17. A method according to any one of claims 1 to 16 wherein the tumor antigen is gp100, CEA or a fragment or modified version of gp100 or CEA.

5 18. A method according to claim 17 wherein the modified gp100 comprises the sequence IMDQVPFSY (SEQ ID NO: 1) and/or YLEPGPVTV (SEQ ID NO:2).

10 19. A method according to claim 17 wherein the modified CEA comprises the sequence shown in Figure 8 (SEQ ID NO:112) and/or YLSGADLNL (SEQ ID NO:113).

FIGURE 1
Monkey #6 (Intranodal Administration)



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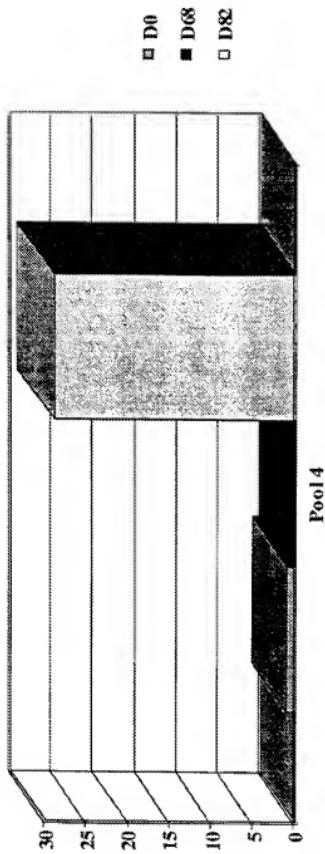
FIGURE 2

Monkey #7 (Intranodal Administration)



FIGURE 3

Monkey # 11 (Subcutaneous Administration)

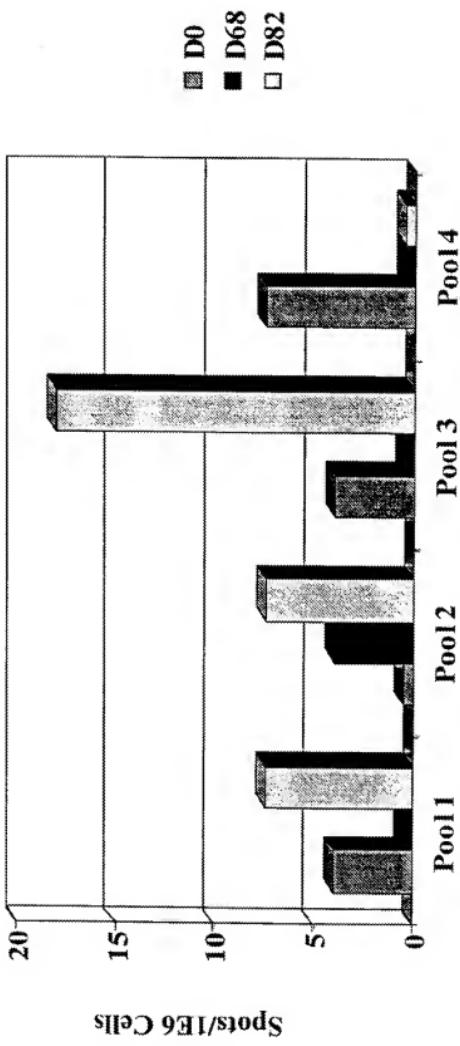


Spots/1E6 Cells

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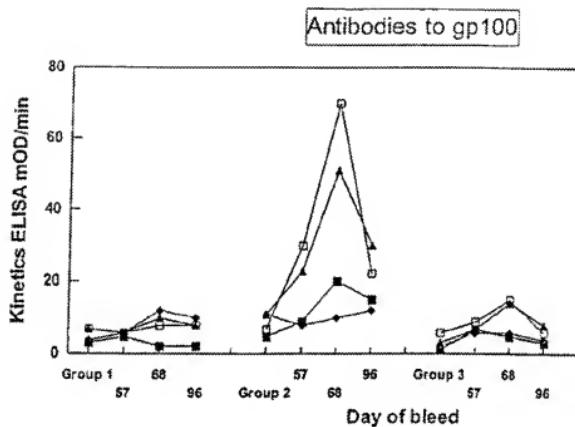
FIGURE 4
Monkey #10 (Subcutaneous Administration)



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FIGURE 5



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FIGURE 6

ATGG ATCTGGTCT AAAAAGATGC CTTCTTCATT TGGCTGTGAT
 AGGTGCTTTG CTGGCTGTGG GGCTCTAAAGA AGTACCCAGA AACCAAGGACT GGCTTGTTGT
 CTCAGGCAAC TCTAGAACCA AAGCCTGAA CAGGCAGCTG TATCCAGAGT GGACAGAAGC
 CCAGAGACTT GACTGCTGGA GAGGTGGTCA AGTGTCCCTC AAGGTGAGTA ATGATGGCC
 TACACTGATT GTGCAAAATG CCTCTTCTCTC TATTCCTCTG AACTTCCCTG GAAGCCAAAA
 GGTTATGCCA GATGGGCAGG TTATCTGGT CAACATACCA ATCATCAATG GGAGCCAGGT
 GTGGGGAGGA CAGCCAGTGT ATCCCCAGGA AACTGACGAT GCCTGCAATCT TCCCTGATGG
 TGGACCTTGC CCATCTGGCT CTTGGCTCA GAAGAGAACG TTGTTATG TCTGGAAGAC
 CTGGGGCAAA TACTGGCAAGG TTCTAGGGGG CCCAGTGTCT GGGCTGAGCA TTGGGACAGG
 CAGGGCAATG CTGGGCACAC ACACGATGGA AGTGAATGTC TACCATGCCG GGGGATCCC
 GAGCTATGTC CCTCTGGCTC ATTCAGCTC AGCCCTCACC ATTATGACCC AGGTGGCTTT
 CTCCGTGAGC GGGTCCCAAGT TGCGGGCCCTT GGATGAGGG AACAAGCCT TCCCTGAGAAA
 TCAGCCTCTG ACCTTTGCCCT TCCAGCTCCA TGACCCCAAGT GCCTATCTGG CTGAGGCTGA
 CCTCTCTCTAC ACCTCTGGCT TTGGAGACAG TGTGGAAACC CTGATCTCTC GGGCACTTGT
 GGTCACTCAT ACTTACCTGG AGCTTGGCCC AGTCACTGTG CAGGTGGTCA TGCAAGGTGC
 CATTCTCTC ACCTCTCTGTG GCTCTCCCC AGTCCAGGG ACCACAGATG GGCACAGGGC
 AACCTCAGAG GCGCCAAACCA CCACAGCTGC CCAAGTGCCT ACTACAGAGG TTGTTGGTAC
 TACACCTGGT CAGGGCCCACT CGCAGAGCC CTCTGGAAACC ACATCTGTGC AGGTGCAAC
 CACTGAAGTC ATAAGCACTG CACCTGTGCA GATGCCAACT CGAGAGACCA CAGGTATGAC
 ACCTGAGAAG GTGCGAGTGT CAGAGGTCTAT GGGTACCACTA CTGGCAGAGA TGTCAACTCC
 AGAGGCTACA GTATGACAC CTGGAGGGT ATCAATTGTG GTGCTTCTG GAACCACAGC
 TGCACAGGTA ACAACTACAG AGTGGTGGG GACCACAGGT AGAGAGCTAC CTATCTCTGA
 GCGTGAAGGT CCAGATGCCA GCTCAATCAT GTCTACGGAA AGTATTACAG GTTCCCTGGG
 CCCCTCTGCTG GTGTTACAG CCACCTTAAG GCTGGTGAAG AGACAAGTCC CCCTGGATTG
 TGTTCTGTAT CGATATGGTT CCTTTTCCGT CACCCCTGGAC ATTGTCACGG GTATTQAAAG
 TGCGGAGATC CTGCAAGGCTG TGCGCTCCGG TGAGGGGGAT GCATTGGAGC TGACTGTGTC
 CTGCCAAGGC GGGCTGCCCA AGGAAGGCTG CATGGAGATC TCATGCCAG GGTGCCAGC
 CCCCTGCCAG CGGCTGTGCC AGCCTGTGCT ACCAGGCCA GCCTGCCAGC TGGTTCTGCA
 CCAGATACTG AAGGGTGGCTT CGGGGACATA CTGCCCTCAAT GTGTCCTCTGG CTGATACAA
 CAGCCTGGCA GTGGTCAGCA CCGAGCTTAT CATGCCCTGGT CAAGAAGCAG GCCTTGGGCA
 GGTTCGGCTG ATCGTGGCA TCTTGGCTGGT GTTGAATGGT GTGGTCCTG CATCTCTGAT
 ATATAAGGCGC AGACTTATGA AGCAAGACTT CTGGTACCC CAGTTGCCAC ATAGCACAG
 TCACTGGCTG CGTCTACCCG GCATCTCTG CTCTTGTCCC ATTGGTGGAGA ACAGCCCCCT
 CCTCACTGGG CAGCAGGTT GA

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FIGURE 7

Met	Asp	Leu	Val	Leu	Lys	Arg	Cys	Leu	Leu	His	Leu	Ala	Val	Ile	Gly
1				5				10						15	
Ala	Leu	Leu	Ala	Val	Gly	Ala	Thr	Lys	Val	Pro	Arg	Asn	Gln	Asp	Trp
				20				25						30	
Leu	Gly	Val	Ser	Arg	Gln	Leu	Arg	Thr	Lys	Ala	Trp	Asn	Arg	Gln	Leu
				35				40					45		
Tyr	Pro	Glu	Trp	Thr	Glu	Ala	Gln	Arg	Leu	Asp	Cys	Trp	Arg	Gly	Gly
				50				55				60			
Gln	Val	Ser	Leu	Lys	Val	Ser	Asn	Asp	Gly	Pro	Thr	Leu	Ile	Gly	Ala
				65				70				75			80
Asn	Ala	Ser	Phe	Ser	Ile	Ala	Leu	Asn	Phe	Pro	Gly	Ser	Gln	Lys	Val
				85							90			95	
Leu	Pro	Asp	Gly	Gln	Val	Ile	Trp	Val	Asn	Asn	Thr	Ile	Ile	Asn	Gly
				100				105				110			
Ser	Gln	Val	Trp	Gly	Gly	Gln	Pro	Val	Tyr	Pro	Gln	Glu	Thr	Asp	Asp
				115				120				125			
Ala	Cys	Ile	Phe	Pro	Asp	Gly	Gly	Pro	Cys	Pro	Ser	Gly	Ser	Trp	Ser
				130				135				140			
Gln	Lys	Arg	Ser	Phe	Val	Tyr	Val	Trp	Lys	Thr	Trp	Gly	Gln	Tyr	Trp
				145				150				155			160
Gln	Val	Leu	Gly	Gly	Pro	Val	Ser	Gly	Leu	Ser	Ile	Gly	Thr	Gly	Arg
				165					170				175		
Ala	Met	Leu	Gly	Thr	His	Thr	Met	Glu	Val	Thr	Val	Tyr	His	Arg	Arg
				180				185				190			
Gly	Ser	Arg	Ser	Tyr	Val	Pro	Leu	Ala	His	Ser	Ser	Ser	Ala	Phe	Thr
				195				200				205			
Ile	Met	Asp	Gln	Val	Pro	Phe	Ser	Val	Ser	Val	Ser	Gln	Leu	Arg	Ala
				210				215				220			
Leu	Asp	Gly	Gly	Asn	Lys	His	Phe	Leu	Arg	Asn	Gln	Pro	Leu	Thr	Phe
				225				230				235			240
Ala	Leu	Gln	Leu	His	Asp	Pro	Ser	Gly	Tyr	Leu	Ala	Glu	Ala	Asp	Leu
				245					250				255		
Ser	Tyr	Thr	Trp	Asp	Phe	Gly	Asp	Ser	Ser	Gly	Thr	Leu	Ile	Ser	Arg
				260				265				270			
Ala	Leu	Val	Val	Thr	His	Thr	Tyr	Leu	Glu	Pro	Gly	Pro	Val	Thr	Val
				275				280				285			
Gln	Val	Val	Leu	Gln	Ala	Ala	Ile	Pro	Leu	Thr	Ser	Cys	Gly	Ser	Ser
				290				295				300			
Pro	Val	Pro	Gly	Thr	Thr	Asp	Gly	His	Arg	Pro	Thr	Ala	Glu	Ala	Pro
				305				310				315			320
Asn	Thr	Thr	Ala	Gly	Gln	Val	Pro	Thr	Thr	Glu	Val	Val	Gly	Thr	Thr
				325					330				335		
Pro	Gly	Gln	Ala	Pro	Thr	Ala	Glu	Pro	Ser	Gly	Thr	Thr	Ser	Val	Gln
				340				345				350			355
Val	Pro	Thr	Thr	Glu	Val	Ile	Ser	Thr	Ala	Pro	Val	Gln	Met	Pro	Thr
				355				360				365			

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FIGURE 7 (CONT'D)

Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
 370 375 380
 Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
 385 390 395 400
 Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
 405 410 415
 Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
 420 425 430
 Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
 435 440 445
 Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
 450 455 460
 Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
 465 470 475 480
 Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
 485 490 495
 Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
 500 505 510
 Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile
 515 520 525
 Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
 530 535 540
 Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
 545 550 555 560
 Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
 565 570 575
 Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
 580 585 590
 Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
 595 600 605
 Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Leu Met Lys Gln Asp
 610 615 620
 Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
 625 630 635 640
 Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
 645 650 655
 Ser Gly Gln Gln Val
 660

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FIGURE 8

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FIGURE 8 (CONT'D)

ACCATTTCCCTCTAAACACATETTAACATGAGTCAGGGGAAATCTGAACTCTCCGCCAC
 721 TGTTAAAGGGGAGATTTGTTGAGATGTCAGTGCCTCTTNGACTTGGAGGGACCGTG 780
 a T I S P L N T S Y R S G E H L N L S C H -
 GCAGCCCTCTAAACCCACCTTGACAGTACTTTGGTTGTCATGGGACTTTCACAGCAATCC
 781 CGTCCGGAGATTGGGTGGACCTGTCATGAGAACCCACAGTACCCCTGAGAAAGGTGTTGG 840
 a A R S N P P A Q Y Q S W F V H N G T F Q Q S -
 ACCCRAGAGCTCTTATCCCAAACATCAGTGTGAAATAGTGGATTCCTTACAGTGCAGA
 841 TGGGTCTCGAAATAAGGGTTGTAOTGACACCTTTATCAGCTAGGATATGACGGGT 900
 a T Q E L F I P N I T V N N S G S Y T C Q -
 GCCCATRACTCTAGAACATGGGCTCTAGAGGACCKAGTCACGGAGATCACAGCTATGAG
 901 CGGGTATTCAGTCAGTGCACCGAGGTTATCCTGGGTCAGTGTCTGAGTGTCAAGATACTC 960
 a A H N S D T G L N R T T V T T I T V Y E -
 CCACCCNACCCCTCTACCCACRGRACAACTCCACCCCGTGGAGGATGAGGATGCTGTA
 961 GGGGGGTTGGAGATGAGTGGTGTGTTGAGGTTGGGGACCTCTACTCCACAGACAT 1020
 a P P K F F I T S N N S N P V E D E D A V -
 GGCCTTACCTCTGAACTCTGAGATTCAGAACACAACTTACCTGTTGGTGGTAATATCAG
 1021 CGGAATTGGACACCTGGACCTCTAAGCTTGTGTTGAGACACCCACCTTATTAGTC 1080
 a A L T C E F E I Q N T T Y L W W V N N Q -
 AGCTCTCCGGTCAGTCCCAAGGCTGAGCTGCAACACAACTTACCTGTTGGTGGTAATATCAG
 1081 TCGGAGGGCCAGTCAGGGCTGGAGCTACAGCTTGTGTTGAGACACCCACCTTATTAGTC 1140
 a S L P V S P R L Q L S N D N R T L T L L -
 AGTGTCAACAGGAATGATGAGTGGACCCCTATGAGTGGAAATCAGAACGGAAATTAAGTGGT
 1141 TCACAGTGTCTTACTACATCCCTGGGAACTACACCTTAAAGGTCTGTTGTTAACTCAGA 1200
 a S V T R N D V G P Y E C G I Q N E L S V -
 GACCAACAGGGACCCAGTCATCCCTGAGTGGCTCTATGGCCCGAGCACCCCAACUATTCC
 1201 CTGGTGTGGCTGGTCAAGTGGACATACAGGAGATAACCCGGTCTGGTGGGGTGSTARAGG 1260
 a D H S D P V I L N V L Y G P S D P T I S -
 CCCCTCATACACCTTATACCCCTGGAGGGTGAACCTCAGGTTCTCTGCCATGAGGCTCT
 1261 GGGAGTATGGATAATGCAAGTCCCACCTTGGAGTGGAGGACGGTACCTCGGAGA 1320
 a P S Y T Y Y R P G V N L S L S C H A R S -
 ARCCCACCTGCAAGTATCTGGCTGATGAGTGGGAACATCCAGAACACACACAGAG
 1321 TTGGGTGGACCTGTCATGAGAACCCACTAATACCTGGTGTAGGTCUTGTCGTTGTC 1380
 a N P P A Q Y Q S W L I D G N I Q Q H T Q S -
 CTCCTTATCTCCACATCACTGAGAAGAACRGCAGGACTCTATACCTGCCAGGCCAATARC
 1381 GAGRAATAGAGGTGAGTGGACTCTCTGCGGCTGAGAATATGGACGGTCCGGTATG 1440
 a L F I S S N I T E K N S G L X T C Q A N N -
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FIGURE 8 (CONT'D)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01253A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used):

MEDLINE, CANCERLIT, LIFESCIENCES, EMBASE, SCISEARCH, EPO-Internal, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47271 A (GUO YAJUN) 18 December 1997 (1997-12-18) page 23, line 14 -page 24, line 22 -----	1-3, 15, 16
X	RAO V S ET AL: "PARTIAL CHARACTERIZATION OF TWO SUBPOPULATIONS OF T-4 CELLS INDUCED BY ACTIVE SPECIFIC INTRALYMPHATIC IMMUNOTHERAPY IN MELANOMA PATIENTS" PROCEEDINGS AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 27, 1986, page 325 XP000990377 ISSN: 0197-016X the whole document ----- -/-	1, 2, 16

 Further documents are listed in the continuation of box C Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/CA 00/01253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>MACKENSEN ANDREAS ET AL: "Homing of intravenously and intralymphatically injected human dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells." CANCER IMMUNOLOGY IMMUNOTHERAPY, vol. 48, no. 2-3, May 1999 (1999-05), pages 118-122, XP000990346 ISSN: 0340-7004 the whole document</p> <p>-----</p>	1-19
A	<p>IRVINE KARI R ET AL: "Recombinant virus vaccination against "self" antigens using anchor-fixed immunogens." CANCER RESEARCH, vol. 59, no. 11, 1 June 1999 (1999-06-01), pages 2536-2540, XP002161590 ISSN: 0008-5472 the whole document</p> <p>-----</p>	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/CA 00/01253

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9747271	A 18-12-1997	AU	727955 B	04-01-2001
		AU	4228397 A	07-01-1998
		CA	2258082 A	18-12-1997
		CN	1221349 A	30-06-1999
		EP	0956046 A	17-11-1999
		JP	11514666 T	14-12-1999